Article

Chinese Science Bulletin

Plant Physiology

October 2012 Vol.57 No.30: 3872–3877
doi: 10.1007/s11434-012-5309-6

Low concentrations of $NaHSO_3$ enhance NAD(P)H dehydrogenase-dependent cyclic photophosphorylation and alleviate the oxidative damage to improve photosynthesis in tobacco

WU YanXia[1†], HE Wei[1†], MA WeiMin[2], SHEN YunKang[1] & MI HuaLing[1*]

[1] *National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China;*
[2] *College of Life and Environment Sciences, Shanghai Normal University, Shanghai 200234, China*

Received February 10, 2012; accepted May 10, 2012; published online July 10, 2012

Bisulfite at low concentrations (*L*-$NaHSO_3$) increases cyclic electron transport around photosystem I (PSI) and photosynthesis. However, little is known regarding the detailed contribution of cyclic electron transport to the promoted photosynthesis by L-$NaHSO_3$. In the present work, we used tobacco mutant defective in *ndhC-ndhK-ndhJ* (*ΔndhCKJ*) to investigate the role of NAD(P)H dehydrogenase (NDH)-dependent cyclic electron transport around PSI in an increase in photosynthesis by L-$NaHSO_3$. After the treatment of tobacco leaves with L-$NaHSO_3$ (10 μmol L^{-1}), the NDH-dependent cyclic electron transport, monitored by a transient post-illumination increase in Chl fluorescence and the amount of NDH, was notably up-regulated in wild type (WT). The NDH-dependent cyclic electron transport was severely impaired in *ΔndhCKJ* and was not significantly affected by treatment with L-$NaHSO_3$. Accordingly, the NDH-dependent transthylakoid membrane proton gradient (ΔpH), as reflected by the slow phase of millisecond-delayed light emission (ms-DLE), was increased by L-$NaHSO_3$ in WT, but not in *ΔndhCKJ*; the enhancement of cyclic photophosphorylation (PSP) activity by L-$NaHSO_3$ was more obvious in WT than *ΔndhCKJ*. The accumulation of both superoxide and hydrogen peroxide was reduced in WT when subjected to L-$NaHSO_3$ treatment, but not in *ΔndhCKJ*. Furthermore, the increase of photosynthetic O_2 evolution rate by L-$NaHSO_3$ was more significant in WT than in *ΔndhCKJ*. We therefore conclude that L-$NaHSO_3$ alleviates the photo-oxidative damage by the enhancement of NDH-dependent cyclic PSP, thereby improving photosynthesis.

bisulfite, NDH-dependent cyclic electron transport around PSI, *Nicotiana tabacum* cv. *Xanthi*, photo-oxidative stress, photosynthesis

Citation: Wu Y X, He W, Ma W M, et al. Low concentrations of $NaHSO_3$ enhance NAD(P)H dehydrogenase-dependent cyclic photophosphorylation and alleviate the oxidative damage to improve photosynthesis in tobacco. Chin Sci Bull, 2012, 57: 3872–3877, doi: 10.1007/s11434-012-5309-6

Bisulfite at low concentrations (L-$NaHSO_3$) can raise net photosynthetic rate and even the yield of crops [1–7]. To investigate the role of L-$NaHSO_3$ in photosynthesis, much work has been carried out. Ziegler and Libera [8] suggested that L-$NaHSO_3$ increases the reduction of dioxygen in PSI to enhance chloroplast CO_2 fixation. Soldatini et al. [9] proposed that L-$NaHSO_3$ causes an increase in thylakoidal sulfolipids, thus enhancing the CO_2 fixation in *Chlorella vulgaris*. Yang et al. [7] indicated that the enhanced net photosynthetic rate by L-$NaHSO_3$ is due to increasing carboxylation efficiency relevant to ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity and regeneration rate of RuBP. However, the mechanism underlying the L-$NaHSO_3$-enhanced photosynthesis is still unclear.

It has been extensively observed that L-$NaHSO_3$ increased the photosynthesis in wheat [5,10], rice [6] and cyanobacterium *Synechocystis* PCC 6803 [11], all of which were accompanied with the evident enhancement of a transient post-illumination increase in Chl fluorescence. Therefore, the increased photosynthesis by L-$NaHSO_3$ is proposed to be relevant to the promotion of the cyclic electron

†These authors contributed equally to this work
*Corresponding author (email: mihl@sippe.ac.cn)

© The Author(s) 2012. This article is published with open access at Springerlink.com

transport around photosystem I (PSI) and cyclic photophosphorylation (PSP). The transient post-illumination increase in Chl fluorescence is considered to arise from the reduction of plastoquinone (PQ) by NAD(P)H catalyzed by NAD(P)H dehydrogenase (NDH) namely NDH-dependent cyclic electron transport around PSI (NDH-pathway) in cyanobacteria [12] and higher plants [13,14]. The NDH knockout plants are sensitive to high light [15], heat [16] and mild desiccation [17]. It has been demonstrated that NDH-dependent cyclic electron transport functions in alleviating oxidative stresses [18]. Recently, we have demonstrated that the NDH-pathway is enhanced by the photo-oxidation of L-$NaHSO_3$ initiated by superoxide anions in PSI [19]. However, little is known regarding the detailed contribution of cyclic electron transport to the promoted photosynthesis by L-$NaHSO_3$.

The aim of the present study is to investigate the physiological significance of NDH-dependent cyclic electron transport enhanced by L-$NaHSO_3$ in photosynthesis. We compared the effects of L-$NaHSO_3$ on the NDH-dependent cyclic electron transport, NDH-dependent cyclic PSP, reactive oxygen species (ROS) accumulation and photosynthesis between the *ndhC-ndhK-ndhJ* defective mutant (*ΔndhCKJ*) and its wild type (WT). We discuss the possible physiological role of NDH-pathway promoted by L-$NaHSO_3$ in photosynthesis.

1 Materials and methods

1.1 Plant materials, growth conditions and treatment

Homoplastic *ΔndhCKJ* tobacco (*Nicotiana tabacum* cv *Xanthi*) mutants in which the chloroplastic *NdhC*, *NdhK*, and *NdhJ* genes were inactivated [20] were cultivated along with wild type plants in a phytotron (about 450 μmol photons m^{-2} s^{-1}, 60% RH, 12-h light at 28°C and 12-h dark at 22°C). All plants received enough organic and compound fertilizer containing sulfate. The plants were watered every 2 d. Fully expanded mature leaves (18–20 cm in length and 10–12 cm in width) in the upper position of the stalk were harvested and the petioles were placed into water in a growth chamber (16-h light at 28°C and 8-h dark at 22°C, 150 μmol photons m^{-2} s^{-1}). The tobacco leaves were treated by infiltrating 100 μL $NaHSO_3$ solution into the abaxial side of the leaves using the blunt end of 1 mL syringe according to Wu et al. [19]. At the times given, the infiltrated sites of leaves were used for analysis.

1.2 Measurements of Chl fluorescence

The kinetics of Chl fluorescence was measured by using a Dual-PAM 100 fluorometer (Walz, Germany). The transient post-illumination increase in Chl fluorescence in tobacco leaves was recorded after termination of actinic light (170 μmol photons m^{-2} s^{-1} for 2 min) at 28°C.

1.3 Thylakoid preparation and protein gel blot analysis

Thylakoid membranes were isolated from fresh, prechilled leaf discs at the infiltrated site by homogenization in STN medium (50 mmol L^{-1} Tris-HCl (pH 7.5), 400 mmol L^{-1} sucrose and 10 mmol L^{-1} NaCl). The homogenate was passed through a nylon cloth (aperture: 100 μm) and then centrifuged at 200×*g* for 3 min to remove the debris. The supernatant was centrifuged at 8000×*g* for 10 min to pellet thylakoid membranes, which were then washed twice and resuspended with TN medium (50 mmol L^{-1} Tris-HCl (pH 7.5) and 10 mmol L^{-1} NaCl). All operations were carried out at 4°C. Then the thylakoid membranes were mixed with SDS loading buffer and incubated in the boiling water for 5 min. Insoluble fractions were removed by centrifugation at 12000×*g* for 5 min, and the supernatant fractions were used for 15% (w/v) SDS-PAGE. Protein gel blot analysis was performed with an ECL Plus assay Kit (Amersham Pharmacia) according to the manufacturer's protocol. The antibodies against NdhH of *Synechocystis* 6803 and NdhJ of rice were raised in our laboratory [19,21].

1.4 Measurements of ms-DLE

Measurements of millisecond-delayed light emission (ms-DLE) were carried out using a phosphoroscope as described [16]. The excitation light was about 2000 μmol photons m^{-2} s^{-1}. The leaves were dark adapted at the room temperature for a few minutes. The 2-cm round discs at the infiltrated site were detached from the leaves and then immediately inserted into the sample cell to measure ms-DLE.

1.5 Measurements of photophosphorylations

Chloroplasts were prepared from fresh tobacco leaves in a similar manner as the thylakoid membranes, but the homogenates were centrifuged at 1000×*g* for 5 min rather than centrifuged at 8000×*g* for 10 min. PSP activity was estimated by measuring light induced ATP synthesis in chloroplasts. ATP production was measured by comparing the ATP level in the dark against the level of 1.5 min after illumination (45 μmol photons m^{-2} s^{-1}) [22] at 28°C. A 1-mL reaction mixture consisted of 50 mmol L^{-1} Tris-HCl (pH 7.6), 10 mmol L^{-1} NaCl, 5 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} ADP, 2 mmol L^{-1} Na_2HPO_4, 0.05 mmol L^{-1} PMS (phenazine methosulfate), chloroplasts (20 μg of Chl), and $NaHSO_3$ at the indicated concentrations. The reaction was terminated by boiling for 3 min. ATP content was then analyzed by the Luciferin-luciferase method using a luminometer (RS 9901 luminometer) and ATP bioluminescence assay kit (Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences).

1.6 Histochemical detection of superoxide and H_2O_2 in the leaves

Superoxide and hydrogen peroxide (H_2O_2) accumulations

were detected by *in situ* staining with nitroblue tetrazolium (NBT) [23] and 3,3-diaminobenzidine (DAB) [24], respectively. The petioles of leaves were placed into water containing 6 mmol L^{-1} NBT or 1 mg mL^{-1} DAB (pH 3.8) and then kept at 22°C in the dark for 3 h to take up the stain. The leaves were then illuminated for 5 h with the petioles immersed in the NBT or DAB solution. The 1-cm round discs at the infiltrated site were detached from the leaves and immediately boiled in 95% ethanol for 10 min to stop the staining and bleach Chl.

1.7 Photosynthetic O_2 evolution

At the indicated times after the $NaHSO_3$ treatment, 1 cm round discs at the infiltrated site were detached from the leaf and cut into small fragments. The fragments were then stirred into a 1.8-mL suspension containing 50 mmol L^{-1} $NaHCO_3$ and 50 mmol L^{-1} Tris-HCl (pH 7.5), in the thermostated glass vessel of a Clark-type oxygen electrode. The photosynthetic O_2 evolution was normally detected several minutes after the beginning of the illumination (800 μmol photons m^{-2} s^{-1}).

2 Results

2.1 Low concentrations of $NaHSO_3$ increase NDH-dependent cyclic electron flow during the transition of the dark to light

The NDH activity, as judged by a transient post-illumination increase in Chl fluorescence (Figure 1(a)), was significantly enhanced by different concentrations of $NaHSO_3$ from 0.1 to 100 μmol L^{-1}, and the maximum increase was observed at 10 μmol L^{-1} in WT [19], therefore, 10 μmol L^{-1} $NaHSO_3$ was chosen for the following experiments. Next, we determined the diurnal pattern of NDH activity of the leaves treated by 10 μmol L^{-1} $NaHSO_3$. No significant change in NDH activity was present over a 5-h time course under light and the subsequent 8 h time course of dark period. However, after the dark-light transition, NDH activity was induced and the enhancement lasted until the end of photoperiod (Figure 1(b)). The NDH activity was impaired in *ΔndhCKJ* and was not significantly affected during the whole photoperiod after the $NaHSO_3$ treatment. Furthermore, we performed immunoblot analysis to check whether L-$NaHSO_3$ affects the translation level of NDH. The accumulation of NdhJ and NdhH was greatly decreased in *ΔndhCKJ* [19]. In accordance with the NDH activity, the expression levels of NdhJ and NdhH in WT were notably up-regulated at 18 h (Hour 18), compared with that at 5 h (Hour 5) after the $NaHSO_3$ treatment (Figure 2). These results indicate that L-$NaHSO_3$ enhances NDH-dependent cyclic electron transport.

2.2 Low concentrations of $NaHSO_3$ increase NDH-dependent cyclic PSP

The millisecond delayed light emission (ms-DLE) of Chl fluorescence originates from the back reaction of PSII. The fast phase of ms-DLE, within 0.1 s at the beginning of the light, is related to the rapid establishment of thylakoid membrane potential. The slow phase following the fast one and reaching a plateau within a few seconds is mainly caused by transthylakoid membrane proton gradient (ΔpH) [25,26]. The slow phase of ms-DLE in WT was higher than *ΔndhCKJ* before the $NaHSO_3$ treatment (Hour 0) (Figure 3), indicating the involvement of NDH-pathway in the formation of ΔpH. After the treatment with 10 μmol L^{-1} $NaHSO_3$, the slow phase of ms-DLE was evidently enhanced in WT by 14% and in *ΔndhCKJ* by 18% at Hour 5, and further

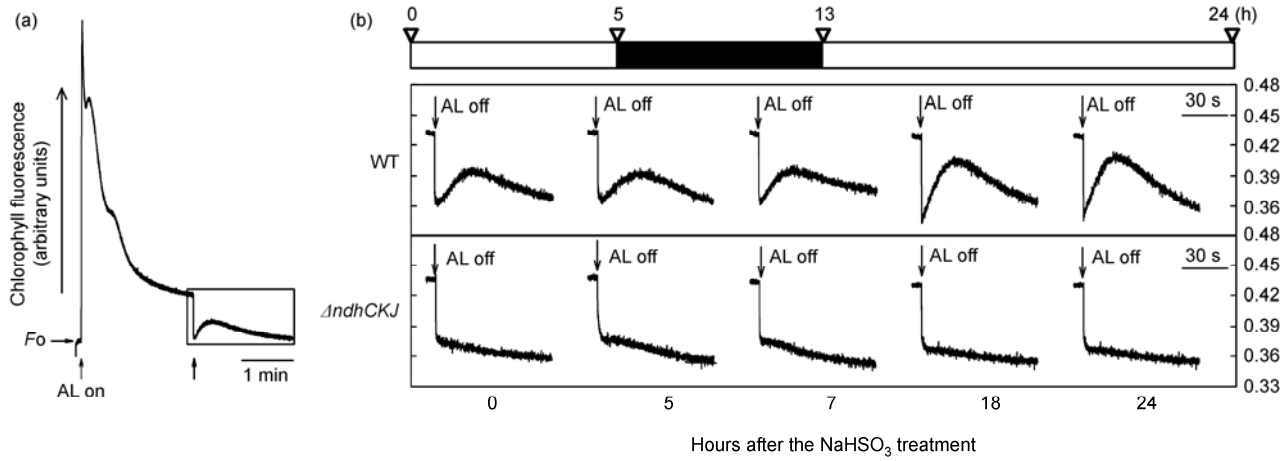

Figure 1 *In vivo* detection of NDH activity by Chl fluorescence measurements. (a) A typical Chl fluorescence kinetics in tobacco leaves of WT. The part in rectangle shows the transient post-illumination increase in Chl fluorescence, which is monitored as an indicator of NDH activity. F_o, dark fluorescence level; AL, red actinic light (170 μmol photons m^{-2} s^{-1}, lasted for 2 min). (b) Diurnal cycle of the NDH activity of the leaves treated by 10 μmol L^{-1} $NaHSO_3$ in WT and *ΔndhCKJ*. The black and white areas of the bar at the top correspond to the dark and light periods of diurnal cycle.

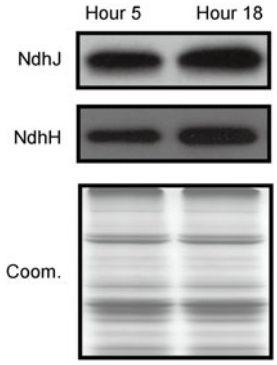

Figure 2 The expression levels of NdhJ and NdhH detected 5 h (Hour 5) and 18 h (Hour 18) after the treatment with 10 μmol L^{-1} $NaHSO_3$ in tobacco leaves of WT. Thylakoid proteins were loaded on an equal Chl basis (7.5 μg). In the lower panel, a replicate gel stained with Coomassie blue (Coom.) is shown as loading control.

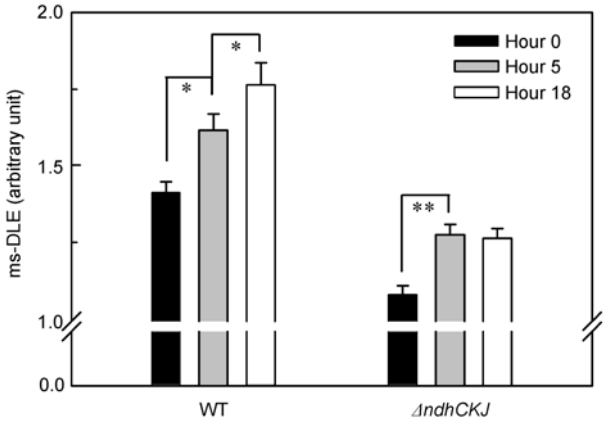

Figure 3 The intensity of slow phase of ms-DLE measured before the $NaHSO_3$ treatment (Hour 0; black bars), 5 h (Hour 5; gray bars) and 18 h (Hour 18; dark gray bars) after the treatment with 10 μmol L^{-1} $NaHSO_3$ in tobacco leaves of WT and *ΔndhCKJ*. Values are means ± SE of five independent measurements. Asterisk indicates significant differences (*t*-test, * $P<0.05$ and ** $P<0.01$).

by 9% at Hour 18 in WT but not in *ΔndhCKJ*. These results indicate that the NDH-dependent ΔpH levels are promoted by L-$NaHSO_3$. To investigate whether the NDH- dependent ΔpH level promoted by L-$NaHSO_3$ contributes to ATP synthesis, the light-induced ATP synthesis in chloroplasts was analyzed. As shown in Figure 4, the photo- induced ATP synthesis in WT was higher than *ΔndhCKJ*, indicating that NDH pathway does contribute to cyclic PSP. The cyclic PSP activity was maximally enhanced by 92% by 100 nmol L^{-1} $NaHSO_3$ in WT and 43% by 1 μmol L^{-1} $NaHSO_3$ in *ΔndhCKJ*. These results confirm the conclusion that L-$NaHSO_3$ enhances NDH-dependent cyclic PSP.

2.3 Low concentrations of $NaHSO_3$ decrease the accumulation of ROS

Since the main function of NDH-dependent cyclic electron

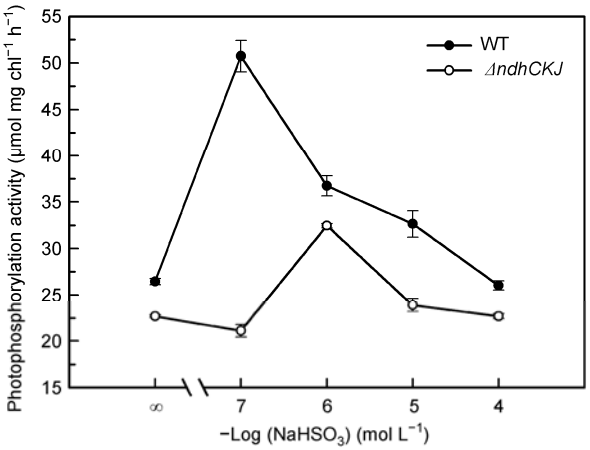

Figure 4 Effects of L-$NaHSO_3$ (0.1–100 μmol L^{-1}) on the light-induced ATP synthesis in tobacco chloroplasts. The 1 mL reaction mixture contained 50 mmol L^{-1} Tris-HCl (pH 7.6), 10 mmol L^{-1} NaCl, 5 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} ADP, 2 mmol L^{-1} Na_2HPO_4, 0.05 mmol L^{-1} PMS, chloroplasts (20 μg of Chl), and $NaHSO_3$ as indicated. ATP content was analyzed using Luciferin-luciferase method. Values are means ± SD from three measurements.

transport is to alleviate the oxidative stress [18], we checked the role of the increased NDH-dependent cyclic electron transport by L-$NaHSO_3$ in removing ROS. Superoxide and H_2O_2 accumulations were detected by *in situ* staining with NBT and DAB, respectively. The accumulation of both superoxide (dark blue mark) and H_2O_2 (brown mark) was greater in *ΔndhCKJ* than in WT (Figure 5). The leaf discs treated by 10 μmol L^{-1} $NaHSO_3$ showed less intense staining of superoxide and H_2O_2 than the leaf discs treated by H_2O in WT at Hour 18, but slightly more intense staining in *ΔndhCKJ*. These results indicate that the L-$NaHSO_3$ functions in scavenging ROS via NDH pathway.

2.4 Low concentrations of $NaHSO_3$ improve photosynthesis

The photosynthetic O_2 evolution of the leaf fragments suspended in a solution of bicarbonate is used to estimate the

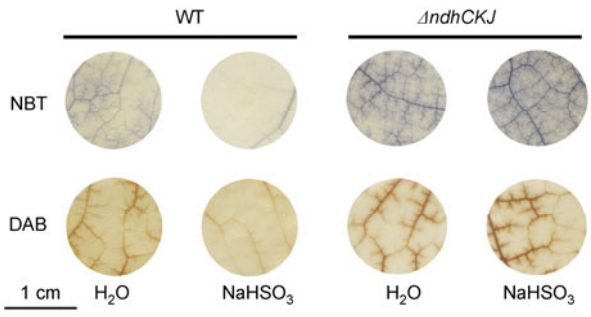

Figure 5 The accumulation of superoxide and H_2O_2 in leaves discs of WT and *ΔndhCKJ* at infiltrated site 18 h after the treatment with 10 μmol L^{-1} $NaHSO_3$ or H_2O, respectively. Superoxide accumulation was detected as dark blue staining by NBT and H_2O_2 as brown staining by DAB. The figure shows representative images from three independent experiments.

activity of CO_2 fixation [16]. The rate of O_2 evolution of WT was higher than *ΔndhCKJ* at Hour 0. After the treatment with 10 μmol L^{-1} $NaHSO_3$, the activity of O_2 evolution was evidently enhanced in WT by 22% and in *ΔndhCKJ* by 37% at Hour 5 and further by 13% at Hour 18 in WT but not in *ΔndhCKJ* (Figure 6). These results indicate that L-$NaHSO_3$ increases photosynthesis via NDH pathway.

3 Discussion

It has been suggested that L-$NaHSO_3$ accelerates the cyclic electron transport to increase the photosynthesis in wheat [5,10], rice [6] and cyanobacterium *Synechocystis* PCC 6803 [11]. However, the mechanism is unclear. In the present study, using the tobacco mutant defective in NDH activity, we clearly show that L-$NaHSO_3$ increases the NDH-dependent cyclic electron transport (Figures 1(b) and 2) and NDH-dependent cyclic PSP (Figures 3 and 4), alleviates the oxidative damage (Figure 5), thereby enhancing the photosynthesis (Figure 6).

In chloroplasts, the main process involved in the formation of ROS is the excitation of oxygen of the triplet state to the singlet state by Chl of triplet excited state in PSII and the photoreduction of oxygen to superoxide radical by reduced electron transport component in PSI [27]. ROS are major damaging factors to the photosynthetic machinery. PSII is vulnerable to ROS, since the rate of the repair of photodamaged PSII is depressed owing to the inhibition of *de novo* synthesis of D1 protein by ROS [28]. In addition to PSII, the reaction center of PSI can also be damaged by ROS, notably under chilling stress [29]. Furthermore, Calvin-Benson cycle enzymes like Rubisco, fructose 1,6-bisphosphatase and ribulose 5-phosphate kinase can be fragmented or inactivated by ROS [30–33]. Thus, ROS can result in a decrease in photosynthesis. Plants have developed a variety of protective mechanisms against oxidative stresses caused by ROS. The cyclic electron transport around PSI has been proposed as an effective route to decrease ROS levels in chloroplasts [28]. It has been observed that NDH-dependent cyclic electron transport is involved in enhancing PSII thermotolerance [34] and photoprotection of PSII [15], probably by the reduction of ROS production. Further, Wang et al. [16] suggested that NDH-dependent cyclic electron transport reduces the ROS contents through the optimization of the photosynthetic apparatus by the enhancement of cyclic PSP. Although there is no apparent phenotype in *ΔndhCKJ* under greenhouse condition, the accumulation of both superoxide and H_2O_2 was greater than that in WT (Figure 5), which is probably responsible for the suppressed photosynthesis in *ΔndhCKJ* (Figure 6). As a result, the increased NDH-dependent cyclic electron transport and cyclic PSP by L-$NaHSO_3$ (Figures 1(b), 2, 3 and 4) reduced the accumulation of ROS (Figure 5), thus enhanced the photosynthesis in WT (Figure 6).

The photosynthesis was also considerably improved by treatment with L-$NaHSO_3$ in both WT and *ΔndhCKJ* at Hour 5, compared with that at Hour 0 (Figures 3 and 6). These results indicate that in addition to the NDH-dependent cyclic electron transport, L-$NaHSO_3$ might also stimulate other regulative pathways, such as Mehler reaction (pseudocyclic electron transport) which also provides ATP for photosynthesis. It is interesting to investigate the role of L-$NaHSO_3$ in regulation of other pathway.

In conclusion, the present results indicate that L-$NaHSO_3$ reduces the accumulation of ROS probably by enhancing the NDH-dependent cyclic electron transport and cyclic PSP, thereby improving photosynthesis.

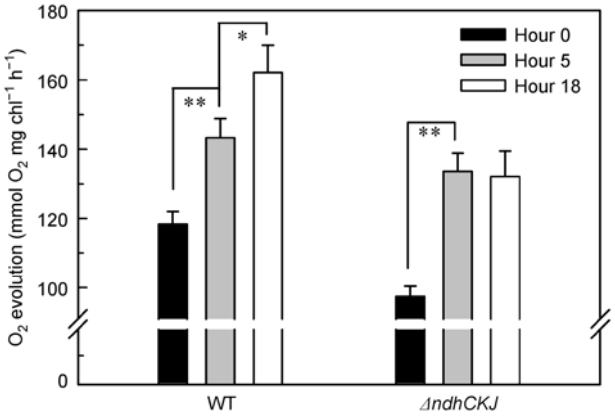

Figure 6 The photosynthetic O_2 evolution rate measured before the $NaHSO_3$ treatment (Hour 0; black bars), 5 h (Hour 5; gray bars) and 18 h (Hour 18; dark gray bars) after the treatment with 10 μmol L^{-1} $NaHSO_3$ in tobacco leaves of WT and *ΔndhCKJ*. Values are means ± SE of five independent measurements. Asterisk indicates significant differences (*t*-test, * $P<0.05$ and ** $P<0.01$).

We appreciate Prof. Toshiharu Shikanai, Kyoto University for providing tobacco mutant ΔndhCKJ. This work was supported by the National Key Basic Research Program of China (2009CB118504) and the National Natural Science Foundation of China (30870183, 31070215).

1 Shen Y, Li D, Wei J, et al. Measurement of photosynthesis with improved dry weight (in Chinese). Plant Physiol Commun, 1980, 2: 37–40
2 Takemoto B K, Noble R D. The effects of short-term SO_2 fumigation on photosynthesis and respiration in soybean *Glycine max*. Envron Pollut, 1982, 28: 67–74
3 Tan S, Shen Y. The effects of sodium bisulfite on photosynthetic apparatus and its operation (in Chinese). Acta Phytophysiol Sin, 1987, 13: 42–50
4 Katainen H S, Mäkinen E, Jokinen J, et al. Effects of SO_2 on the photosynthetic and respiration rates in Scots pine seedlings. Environ Pollut, 1987, 46: 241–251
5 Wang H, Wei J, Shen Y. Enhancement of wheat leaf photophosphorylation and photosynthesis by spraying low concentrations of $NaHSO_3$ (in Chinese). Chin Sci Bull (Chin Ver), 2000, 45: 1308–1311
6 Wang H, Wei J, Shen Y, et al. Enhancement of photophosphorylation and photosynthesis in rice by low concentrations of $NaHSO_3$ under field conditions. Acta Bot Sin, 2000, 42: 1295–1299
7 Yang W, Chen H, Zhu F, et al. Low concentration of bisulfite en-

hances photosynthesis in tea tree by promoting carboxylation efficiency in leaves. Photosynthetica, 2008, 46: 615–617
8 Ziegler I, Libera W. The enhancement of CO_2 fixation in isolated chloroplasts by low sulfite concentrations and by ascorbate. Z Naturforsch, 1975, 30c: 634–637
9 Soldatini G F, Ziegler I, Ziegler H. Sulfite: preferential sulfur source and modifier of CO_2 fixation in *Chlorella vulgaris*. Planta, 1978, 143: 225–23l
10 Wang H, Su J, Shen Y. Difference in response of photosynthesis to bisulfite between two wheat genotypes. J Plant Physiol Mol Biol, 2003, 29: 27–32
11 Wang H, Mi H, Ye J, et al. Low concentrations of $NaHSO_3$ increase cyclic photophosphorylation and photosynthesis in cyanobacterium *Synechocystis* PCC6803. Photosynth Res, 2003, 75: 151–159
12 Mi H, Endo T, Ogawa T, et al. Thylakoid membrane-bound, NADPH-specific pyridine nucleotide dehydrogenase complex mediates cyclic electron transport in the cyanobacterium *Synechocystis* sp. PCC6803. Plant Cell Physiol, 1995, 36: 661–668
13 Burrows P A, Sazanov L A, Svab Z, et al. Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid *ndh* genes. EMBO J, 1998, 17: 868–876
14 Shikanai T, Endo T, Hashimoto T, et al. Directed disruption of the tobacco *ndhB* gene impairs cyclic electron flow around photosystem I. Proc Natl Acad Sci USA, 1998, 95: 9705–9709
15 Endo T, Shikanai T, Takabayashi A, et al. The role of chloroplastic NAD(P)H dehydrogenase in photoprotection. FEBS Lett, 1999, 457: 5–8
16 Wang P, Duan W, Takabayashi A, et al. Chloroplastic NAD(P)H dehydrogenase in tobacco leaves functions in alleviation of oxidative damage caused by temperature stress. Plant Physiol, 2006, 141: 465–474
17 Horváth E M, Peter S O, Joët T, et al. Targeted inactivation of the plastid *ndhB* gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure. Plant Physiol, 2000, 123: 1337–1350
18 Shikanai T. Cyclic electron transport around photosystem I: Genetic approaches. Annu Rev Plant Biol, 2007, 58: 199–217
19 Wu Y, Zheng F, Ma W, et al. Regulation of NAD(P)H dehydrogenase-dependent cyclic electron transport around photosystem I by $NaHSO_3$ at low concentrations in tobacco chloroplasts. Plant Cell Physiol, 2011, 52: 1734–1743
20 Takabayashi A, Endo T, Shikanai T, et al. Post-illumination reduction of the plastoquinone pool in chloroplast transformants in which chloroplastic NAD(P)H dehydrogenase was inactivated. Biosci Biotechnol Biochem, 2002, 66: 2107–2111
21 Ma W, Mi H. Expression and activity of type-1 NAD(P)H dehydrogenase at different growth phases of cyanobacterium, *Synechocystis* PCC6803. Physiol Plant, 2005, 125: 135–140
22 Wei J, Shen Y, Li D, Xu C. Stimulatory effect of sodium bisulfite on cyclic photophosphorylation of chloroplasts under low light intensity (in Chinese). Acta Phytophysiol Sin, 1989, 15: 101–104
23 Fryer M, Oxborough K, Mullineaux P, et al. Imaging of photo-oxidative stress responses in leaves. J Exp Bot, 2002, 53: 1249–1254
24 Thordal-Christensen H, Zhang Z, Wei Y, et al. Subcellular localization of H_2O_2 in plants: H_2O_2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. Plant J, 1997, 11: 1187–1194
25 Li D, Shen Y. The relationship between components of proton motive force and photosynthesis (in Chinese). Chin Sci Bull (Chin Ver), 1994, 39: 1712–1715
26 Wraight C A, Crofts A R. Delayed light emission and high energy state of chloroplasts. Eur J Biochem, 1971, 19: 386–397
27 Asada K. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol, 2006, 141: 391–396
28 Takahashi S, Badger M R. Photoprotection in plants: A new light on photosystem II damage. Trends Plant Sci, 2011, 16: 53–60
29 Sonoike K. Photoinhibition of photosystem I: Its physiological significance in the chilling sensitivity of plants. Plant Cell Physiol, 1996, 37: 239–247
30 Kaiser W. Reversible inhibition of the Calvin cycle and activation of oxidative pentose phosphate cycle in isolated intact chloroplasts by hydrogen peroxide. Planta, 1979, 145: 377–382
31 Tanaka K, Otsubo T, Kondo N. Participation of hydrogen peroxide in the inactivation of Calvin-cycle SH enzymes in SO_2-fumigated spinach leaves. Plant Cell Physiol, 1982, 23: 1009–1018
32 Ishida H, Nishimori Y, Sugisawa M, et al. The large subunit of ribulose-1,5-bisphosphate into 37-kDa and 16-kDa polypeptides by active oxygen in the lysates of chloroplasts from primary leaves of wheat. Plant Cell Physiol, 1997, 38: 471–479
33 Ishida H, Shimizu S, Makino A, et al. Light-dependent fragmentation of the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase in chloroplasts isolated from wheat leaves. Planta, 1998, 204: 305–309
34 Rowland J G, Simo W J, Nishiyama Y, et al. Differential proteomic analysis using iTRAQ reveals changes in thylakoids associated with Photosystem II acquired thermotolerance in *Synechocystis* sp. PCC 6803. Proteomics, 2010, 10: 1917–1929

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.